#  *Simplexa Bordetella* PCR Assay Procedure

**PURPOSE**

* This procedure provides instructions for preparing samples, setting up the PCR reaction and running the *Simplexa Bordetella* PCR assay for the detection of *B. pertussis and B. parapertussis* from nasal and bronchial specimens

#### POLICY STATEMENT

* PCR testing is performed daily, 0700 –1530

**ABBREVIATIONS**

1. BOR: *Bordetella*
2. BORDP: *Bordetella* PCR
3. Bp: *Bordetella pertussis*
4. Bpp: *Bordetella parapertussis*
5. BSC: BioSafety Cabinet
6. BSL: BioSafety level
7. CFU: colony forming unit
8. Ct: crossing threshold
9. F/T: freeze/thaw
10. IC: internal control
11. MM: master mix
12. NA: Nucleic Acid
13. NEGC: negative control
14. NFW: nuclease free water
15. PCR: polymerase chain reaction
16. PCTL: process control
17. POSC: positive control
18. PP: primer – pair
19. PPE: personal protective equipment
20. SEAC: Simplexa extraction and amplification control
21. TE buffer: Tris – EDTA buffer
22. UNAC: Specimen unacceptable, please recollect

## DOCUMENTATION/RECORDS

1. Simplexa run-specific Segment Report
2. LIS Incomplete and Completed worksheets
3. Daily Maintenance Log

## SAFETY CONSIDERATIONS

1. Standard precautions for infectious agents. Refer to [MB002.2](file:///G%3A%5CLAB%5CMolecular%20Biology%5CA.%20Molecular%20Procedure%20Manual%5CMB002%20Safety%5CMB%20002.2%20v4%20Biohazard%20Containment.docx), Biohazardous containment
2. Use of engineering controls: Refer to [MB003.1](file:///G%3A%5CLAB%5CMolecular%20Biology%5CA.%20Molecular%20Procedure%20Manual%5CMB003%20Engineering%20Controls%5CMB%20003.1%20Engineering%20Controls%20to%20Prevent%20Nucleic%20Acid%20Contamination.doc) Engineering Controls to Prevent Nucleic Acid Contamination
3. General Safety: [MB 002.1](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLAB%5CMolecular%20Biology%5CA.%20Molecular%20Procedure%20Manual%5CMB002%20Safety%5CMB%20002.1%20Safe%20Work%20Practices%20in%20Molecular.doc) Safe Work Practices
4. *Caution:* PPE including protective eyewear must be worn when working with concentrated Extran

#### MATERIALS REQUIRED

| **Equipment** | **Reagents** | **Supplies** |
| --- | --- | --- |
| Room 1: Clean room* Laminar-flow hood, Clean rm 1
* Freezer, -10 to -30⁰ C
* Refrigerator, 2 to 8⁰ C
* Microcentrifuge
* Nalgene cooling block
* Vortex
* Eppendorf Repeater pipette
* Dedicated set of pipettes: 2 µl, 10 µl, 20 μl, 100 μl, 200 μl, and 1000 μl pipettes

Room 2: Processing* BSC, Process rm 2
* Refrigerator, 2 to 8⁰ C
* Freezer, ≥ - 70⁰C
* Nalgene cooling block
* Vortex
* Microcentrifuge
* Dedicated set of pipettes: 2 µl, 10 µl, 20 μl, 100 μl, 200 μl, and 1000 μl pipettes
* Gilson Concept pipette, 100 µl

Room 3: Amplification and detection* Focus Simplexa Integrated Cycler

Room: Microbiology* McFarland densitometer (micro)
 | TE buffer | Micro tube racks |
| Nuclease Free Water (NFW) | 2 ml cryovials |
| SEAC* Internal control PP
* Internal control DNA
 | Sterile filtered pipette tips for 10 µl, 20 µl, 100 μl, 200 µl, 1000 µl pipettes |
| Bp PP | Micro tubes 1.5 ml, RNase/DNase free |
| Bpp PP | Nitrile gloves (powder-free) |
| Bordetella Molecular Control (POSC) | Sharps disposal container  |
| Bordetella process control (PCTL) | Gripper rack, rm 2 |
| TA MasterMix | Orange barrier wipes |
| Sani-Cloth Bleach wipes | BBL™CultureSwab™ |
| 70% alcohol | 12X75 sterile plastic test tubes |
| 5% Extran | Sterile Q – Tipped applicator swabs |
| *Bordetella pertussis* ATCC 8467 | 50 ml sterile conical tube |
|  | Eppendorf 5 ml tips |
|  | Serological pipettes, 5 and 10 ml |
|  | Sterile scissors |
|  |  |

## QUALITY CONTROL

1. Assay Controls
	1. A PCTL, POSC and NEGC must be included in each assay run.
	2. An IC is incorporated into each reaction mixture.
2. QC Monitors:

|  |  |
| --- | --- |
| **Control** | **Control Monitor** |
| Positive Control (POSC) | Reagent failure and primer-probe integrity |
| Negative Control (NEGC) | Reagent and/or environmental contamination, cumulative effect  |
| Process Control (PCTL) | Elution and/or lysis failure; cross contamination; reagent failure |
| Internal Control (IC) | PCR inhibition in specimen, reagent failure or process error |

1. Before reporting patient results, all controls must yield valid results. Refer to BOR 005, Procedures F and G, Evaluating and Interpreting Results.

**PROCEDURE A:** Follow the steps in the table below to prepare specimens for testing

Testing Preparation

| **Activity** | Step | **Action** | **Related Doc** |
| --- | --- | --- | --- |
|  | 1 | Call worksheet **BORDP**; use this worksheet for sample identification throughout testing. | [MB001.1](file:///G%3A%5CLAB%5CMolecular%20Biology%5CA.%20Molecular%20Procedure%20Manual%5CMB001%20Specimen%20Management%5CMB001.1%20Specimen%20Management%20in%20Molecular.doc) Specimen Management |
| **Sample Order****Room 2** | 2 | Process patient samples plus one POSC, NEGC and PCTL per run. Position samples and controls as follows:

|  |  |
| --- | --- |
| Sample | Position |
| Patient samples | 1 – nn |
| PCTL | 3rd to last position |
| POSC | 2nd to last position |
| NEGC | Last tube |

 | [MB003.1](file:///G%3A%5CLAB%5CMolecular%20Biology%5CA.%20Molecular%20Procedure%20Manual%5CMB003%20Engineering%20Controls%5CMB%20003.1%20Engineering%20Controls%20to%20Prevent%20Nucleic%20Acid%20Contamination.doc) Engineering Controls[MB 002.1](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLAB%5CMolecular%20Biology%5CA.%20Molecular%20Procedure%20Manual%5CMB002%20Safety%5CMB%20002.1%20Safe%20Work%20Practices%20in%20Molecular.doc)Safe Work Practices |
| **Organizing run****Room 2** | 3 | Using the BORDP worksheet as a layout, organize patient specimens and labels

|  |  |
| --- | --- |
| Step | Action |
| a | Color code worksheets and labels per run |
| b | Number patients on worksheet in consecutive order |
| c | Number corresponding patient labels according to assigned numbers on worksheet, color coded by run |
| d | Number each primary patient specimen according to worksheet |

 |  |
| **Process NP swabs** | 4 | Prepare NP swabs for testing

|  |  |
| --- | --- |
| Step | Action |
| a | Number cap of a 200 µl TE tube according to assigned number on worksheet |
| b | Properly label TE tube with patient aliquot label matching the number on the cap to the number on the label |
| c | Verify number on primary and secondary container before transfer |
| d | Cut the wire mini-tip swab into the TE buffer tube with corresponding number on cap |
| e | Vortex 5 min, vortex setting 9 |

 |  |
| **Process Bronchs, nasal washes/aspirates** | 5 | Number and label a 2.0 ml cryovial for each nasal wash/aspirate and bronch specimen to be tested

|  |  |
| --- | --- |
| Step | Action |
| a | Number cap of each cryovial according to assigned number on worksheet |
| b | Properly label the tube with patient aliquot label matching the number on the cap to the number on the label |
| c | Vortex specimen in original container until well mixed  |
| d | Verify number on primary and secondary container before transfer  |
| e | Transfer specimen into tube with corresponding number on cap* Only one tube can be open at a time
 |

 |  |
|  | 6 | Place numbered tubes in consecutive order in gripper rack |  |
|  | 7 | Change gloves when possible contamination is suspected or every 8 samples |  |

**PROCEDURE B:** Follow the steps in the table below for setting up the computer

Computer set-up

|  |  |  |  |
| --- | --- | --- | --- |
| **Activity** | Step | **Action** | **Related Doc** |
| **Computer Set-up****Room 3** | 1 | Set up Simplexa; take run specific patient labels into room 3

|  |  |  |
| --- | --- | --- |
| Step | Prompt | Action/Entry |
| a | ------ | Turn on the Simplexa Integrated Cyclers (ABC) |
| b | ------ | Turn on the Simplexa computer |
| c | ------ | Log on computer |
| d | User name | administrator |
| e | Password | focusIC#1 |
| f | ------ | Double-click on Integrated Cycler icon DX icon to open program |
| g | User name | Enter personal user code  |
| h | Password | Enter personal password code  |
| i | ----- | Select **Setup Run** from Quick pick list |
| j | Assay definition | Select **BORD** from drop down box |
| k | Run Name Prefix | **BORD** |
| l | Lot information | PP lot: Add/deactivate reagent lot numbers as needed |
| m | Add Samples | Scan barcode ID from each label consecutively |
| n | Controls | Assign controls according to layout |
| o | ----- | Click **Move to Disc** button |
| p | ----- | Click **Save** to save the run for later use *or*  |
| q | ----- | Click **Run** to save the run and open the **Start Run** window |
| r | ----- | (Optional) Click the **Print Preview** button to generate a layout report, refer to Fig.1 |
| s | ----- | Recycle labels when run is complete; do not take back to room 2 |

 |  |
| **New user** | 2 | To switch users: Select **File: Switch Users***Note*: Users cannot be changed during a run |  |
| **Delete or Edit Segment** | 3 | To delete or edit segments, right click one of the wells in the segment

|  |  |
| --- | --- |
| Step | Action |
| a | Select action: Edit Segment or Delete Segment* Delete Segment will remove all test samples from run
* Edit Segment will move samples from the disc back to the sample list where changes can be made
 |
| b | To move samples back to disc, click starting well location in Disc View |
| c | Click **Move to Disc** button |

 |  |
| **Change PPE** | 4 | Remove lab coat |  |
|  | 5 | Change gloves; move to room 1 |  |

**Figure 1:** Spoke 1 isidentified by theopen slot on the outer ring of the disc. The wells are

 identified from the outer–edge inward A – H. Numerical assignment of the wells is in vertical order.

|  |  |
| --- | --- |
|  |   |

**PROCEDURE C:** Follow the steps in the table below for preparing the MM

Master Mix preparation

| **Activity** | Step | **Action** | **Related Doc** |
| --- | --- | --- | --- |
| **Thaw/warm reagents****Room 1** | 1 | Remove MM components from –20° C freezer/refrigerator; warm to room temperature (approx 15 min) protected from light; use within 1 h |  |
|  | 2 | Gently mix each MM component prior to each use; briefly centrifuge* Larger volumes: Vortex 2 – 3 sec, setting 8 (IC DNA and TA MM)
* Lower volumes: flick tube 4 – 5 times (IC, Bp and Bpp PP)
* Centrifuge: 1 – 2 sec
 | Refer to MM chart [BOR 004](BOR%20004%20Reagent%20and%20Control%20Preparation.docx) |
| **MasterMix** | 3 | Prepare MM in 1.5 micro-centrifuge tube according to chart volumes |  |
| **Room 1** | 4 | Gently vortex MM; briefly centrifuge* Vortex setting: 8
* Time: 2 sec
* Centrifuge: 1 – 2 sec
 |  |
|  | 5 | Return reagents to refrigerator, do not refreeze | [BOR 003](BOR%20003%20Storage%20%26%20Stability%20of%20Processed%20Sample%20%26%20Reagents.docx) Storage and Stability |
|  | 6 | Proceed to PCR set-up |
|  | 7 | Remove lab coat; move to room 2 |  |
| **Room 2** | 8 | Place MM in cooling block until use |  |
|  | 9 | *Keep MM protected from light. Use MM within 30 min of preparation* |  |

**PROCEDURE D:** Follow the steps in the table below for PCR set-up and amplification

**PCR set-up and amplification**

| **Activity** | Step | **Action** | **Related Doc** |
| --- | --- | --- | --- |
| **Vortex****Room 2** | 1 | Vortex specimen tubes prior to set-up if they have been sitting for more than 30 min after initial processing |  |
|  | 2 | Remove Universal disc from package and set on disc cold block |  |
| **Load MM**  | 3 | Position spoke 1 over silver plate groove (refer to Fig. 1) |  |
| **Room 2** | 4 | Pipette 7 µl of MM into each well to be used

|  |  |
| --- | --- |
| ***Tip*** | * Automatic pipettor: hold at slight angle to maintain accuracy
 |
| * Manual pipetting: hold the pipette at a 30-degree angle inserting the tip under the roof of the well to reduce possible contamination

  |

 | [Simplexa Operator Manual](file:///G%3A%5CLAB%5CMolecular%20Biology%5CA.%20Molecular%20Procedure%20Manual%5CMolecular%20Resources%5CSimplexa%20Operator%20Manual%20PI.MOL1101.UD_REV.F.pdf) |
| **Load samples** | 5 | Slowly pipette 3 µl of each patient sample and each control into appropriate well* PCTL: undiluted
* POSC: undiluted
* NEGC: NFW

*Caution*: Do not go to second stop to avoid introduction of bubbles and producing aerosols |  |
|  | 6 | Apply the cover tape on the disc in horizontal position |  |
| **Seal disc** | 7 | Use the disc applicator to seal the cover tape  |  |
|  | 8 | Remove cover tape tabs by gently pulling outwards |  |
| **Change gloves** | 9 | Remove lab coat  |  |
|  | 10 | Change gloves; move to room 3 |  |
| **Room 3** | 11 | Place disc into the instrument; close lid |  |
| **Start Run** | 12 | Click **Run** button to move to status screen |  |
|  | 13 | Select test instrument from drop down box |  |
|  | 14 | Click **Start***Note:* Once the run is started, it cannot be canceled and then restarted using the same disc. Canceling will require a new disc. |  |
|  | 15 | Remove lab coat  |  |
| **Change gloves** | 16 | Change gloves before leaving room 3 |  |
|  | 17 | Approximate run time: 1 h |  |
|  | 18 | Run progress can be viewed in the **Run Status Window**: refer to Fig. 2 |  |
|  | 19 | Remove disc from instrument; *check well volumes for pipetting accuracy* |  |
| **Run completion** | 20 | Place in bio-bag |  |
|  | 21 | Discard in red biohazard container |  |

**Figure 2**: The graph plots detection progress in Real-Time

Dye drop down box for Bp (FAM), Bpp (CFR610) and IC (Q670)

Amplification curve

 (Data acquisition)

Instrument drop down box

Progress bar shows estimated end time

**PROCEDURE E:** Follow the steps in the table below for analysis of data

Analyzing Completed Runs

| **Activity** | **Step** | **Action** | **Related doc** |
| --- | --- | --- | --- |
| **Analyze Results** | 1 | Click the Analyze button at the bottom of the screen to open the Analysis Window |  |
| **Summary** | 2 | Click on the run Details tab to display a summary of the run, target Ct and IC Ct values |  |
|  |  |  |  |
| Room 3**Review amplification curves** | 3 | Review IC Ct results and amplification curves for exponential growth and possible inhibition or low target amplification, refer to Figures 3 and 4

|  |  |
| --- | --- |
| Step | Action |
| a |  Select **Data** tab |
| b | Click on **Print Preview** |
| c | Check **Include Graphs** |
| d | Scroll through the report , reviewing comments, failures and amplification curves |
| e | A valid curve shows a smooth, exponential increase |
| f | Invalid curve may be linear or a curve with data “spikes” where the curve crosses the threshold |
| g | If curve is valid, the Ct values may be used to interpret the results |
| h | Confirm results by a second reviewer before releasing  |
| I | Positive results: Confirm name and accession number on primary sample/TE buffer before releasing |
| j | Select or deselect results to be released |

 | Refer to procedures F, G and H for interpretation of QC and patient results and Exporting results to LIS |
| **Analysis Window**Review channels by clicking dye box(es) to be reviewedData / Detail tabs |   4 | **Figure 3:** Analysis WindowExport drop downSelect and Deselect buttonsPrint Preview |  |
| **Curve examples** | **5** | **Figure 4:** Valid and invalid amplification curves **Valid Valid Invalid** |  |

**PROCEDURE F:** Follow the activities below for evaluating QC acceptability

**Evaluating and Interpreting QC Results**

| **Activity** | **Step** | **Action** | **Related doc** |
| --- | --- | --- | --- |
|  | 1 | Check QC to determine if the run is valid before reporting patient results |  |
|  | 2 | Failure indications will be highlighted in yellow

|  |  |
| --- | --- |
| Step | Action |
| a | Click the Print Preview button to review the “Data Quality message” on the Segment report under QC Notes |
| b | Review associated amplification curves and Ct values |
| c | Click the **Print** button to generate a report for the **QC Error** Log documentation |
| d | Record corrective action on QC log |
| e | Record number of failed tests on **Failed Run** log |

 | Simplexa Operator Manual[Appendix B: Troubleshooting](file:///G%3A%5CLAB%5CMolecular%20Biology%5CA.%20Molecular%20Procedure%20Manual%5CMolecular%20Resources%5CSimplexa%20Troubleshooting%20guide%2C%20Appendix%20B.pdf) |
| QC / Valid assay | 3 | For a valid run, the following QC conditions must be met:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Control | Bp Ct | Bpp Ct | IC Ct | Assay Result |
| POSC | 24 – 34  | 20 - 30 | NA | Positive |
| PCTL | 28 – 34  | 0 | NA | Positive |
| NEGC  | 0 | 0 | 20 – 34  | Negative |

 | 3SD ranges periodically determined in EP Evaluator and programmed into the Simplexa |
| **QC conditions not met****Invalid assay** | 4 | If | Then | Refer to BOR 005, Proc. I for repeat testing |
| **Valid assay:** Controls as expected | * Report patient results
 |
| **Invalid assay:**PCTL/POSC/ NEGC failure | * Do not report patient results
* Repeat patient testing
 |
| PCTL negative or out of range | * Review the specimen handling/ preparation technique
* Repeat patient testing
 |
| POSC negative or out of range | * Review the specimen handling/ preparation technique
* Repeat patient testing
 |
| NEGC positive | * Possible contamination of samples
* Review the specimen handling/ preparation technique
* Repeat patient testing
 |
| IC fails (not detected in the NEGC) | * Failure caused by reagent or system failure
* Repeat patient testing
 |
| PCTL/POSC/ NEGC are invalid  | * Failure caused by inhibition, reagent or system failure
* Repeat patient testing
 |
|  |  | Problem unresolved | * Call Focus technical service, **1-800-838-4548, option 3**
* Notify Technical Specialist or Technical Director
 |  |
| **Problem Log** | 5 | Do not report patient results until problem is resolved |  |
|  | 6 | Record problem/operator action in the QC failure log |  |

**PROCEDURE G:** Follow the activities below for evaluating the acceptability of patient results

**Evaluating and Interpreting Patient Results**

|  |  |  |  |
| --- | --- | --- | --- |
| **Activity** | **Step** | **Action** | **Related doc** |
| Patient Results | 1 | Review amplification curves for each result for exponential growth and data spikesReview “QC statement/Note” on the Segment Report for failures* Document operator action for failures on QC log and Segment report
 | Refer to Fig. 3, 4 |
|  | 2 | If the amplification curve is valid, use Ct value to determine if Bp or Bpp was detected |  |
|  | 3 | Patient results will be reported as *Positive* or *Negative* for Bp and Bpp |  |
| **Internal Control** | 4 | **If** | **Then** | BOR 007Reporting and Archiving BORDP Results |
| IC is detected | * Negative results are valid
* Positive results are valid
 |
| IC is not detected | * Negative results are invalid
* If the Bp or Bpp amplification curves are positive, the IC is not required to be detected ; positive result valid
 |
| Invalid result | * Failure caused by inhibition: Extract 200 µl sample on the EasyMag (RVP protocol); repeat testing from eluate
* Reagent or system failure: Repeat testing from original sample
* If repeat testing remains unresolved, report UNAC
 |
|  | 5 | Refer to **Table 1** for interpretation of results. |  |

###### Table 1: Interpretation of Patient Results: Refer to BOR 007 and BOR 007.A1 for *Reporting and Archiving Patient Results*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Scenario | Bp Ct value | Bpp Ct value | IC Ct value | Interpretation |
| 1 | 0 | 0 | 20 – 34  | Bp and Bpp negative |
| 2 | 13 – 39  | 0 | 0 – 40  | Bp positive, Bpp negative |
| 3 | 0 | 13 – 39  | 0 – 40  | Bpp positive, Bp negative |
| 4 | 13 – 39  | 13 – 39  | 0 – 40  | Bp and Bpp positive |
| 5 | 0 | 0 | 0 | Invalid: repeat |

**PROCEDURE H:** Follow the steps in the table below for exporting data to LIS from the analysis screen

Exporting Data to LIS

| **Activity** | Step | **Action** | **Related Doc** |
| --- | --- | --- | --- |
| **Select data** | 1 | If all test results were valid upon review, click the Select **√** onthe **Data** tab to be exported *or* | BOR 007Reporting and Archiving Results |
|  | 2 | Select the tests individually by placing a check in each box adjacent to the accession number *Note:* Do not export PCTL, POSC and NEGC or failed patient results |  |
| **Export** | 3 | From the Export drop down box, select **LIS** and then **LIS folder;** click **OK**  |  |
|  | 4 | A message that the run exported successfully will appear. Click **OK** |  |
| **Print Report****(Optional)** |  | Print report after review (optional) Fig. 3

|  |  |
| --- | --- |
| Step | Action |
| a | Click **Print Preview** button for multi-page analysis report |
| b | Checkbox: **Include Graphs**  |
| c | Scroll from page to page using the arrow buttons at the top of the screen |
| d | **Print** |

 |  |

**PROCEDURE I:** Follow the activities below for repeat testing

**Repeat Testing**

| **Activity** | **Step** | **Action** | **Related doc** |
| --- | --- | --- | --- |
|  | 1 | Perform repeat testing from original specimen aliquot or TE buffer tube  | Refer toBOR 005, Proc. D |
| **Timeframe** | 2 | Repeat within 5 day if stored at 2 – 8⁰ C |  |
|  | 3 | Repeat samples may be retested in the same run as new samples |  |
| **Vortex** | 4 | Vortex the specimen tubes prior to retesting; vortex setting 9 |  |
| Type of Failure | 5 | Review type of failure (not all inclusive)

|  |  |
| --- | --- |
| Failure | Action |
| Inhibition | * Extract 200 µl on EasyMag (RVP protocol); test eluate
* If sample remains unresolved, call caregiver for new collection
 |
| PCTL  | * Vortex PCTL and specimen tubes; repeat testing
* If PCTL fails on repeat, thaw new PCTL
 |
| POSC/NEGC | * Repeat run from patient aliquot or TE buffer tubes
* Replace NEGC if contamination is indicated
* Pipette carefully to avoid possible aerosol contamination
* Vortex POSC and specimen tubes; repeat testing
* If POSC fails on repeat, thaw new POSC
 |
| System error | * Repeat run from patient aliquot or TE buffer tubes including PCTL/POSC/NEGC
 |
| Failure unresolved | * Call Focus technical service, **1-800-838-4548, option 3**
* Notify Technical Specialist or Technical Director
 |

 | [Simplexa Operator Manual](file:///G%3A%5CLAB%5CMolecular%20Biology%5CA.%20Molecular%20Procedure%20Manual%5CMolecular%20Resources%5CSimplexa%20Troubleshooting%20guide%2C%20Appendix%20B.pdf)Appendix B: Troubleshooting[BOR 006](BOR%20006%20Troubleshooting%20guide.docx) Troubleshooting Guide |

**PROCEDURE J:** Follow the steps in the table below for Simplexa instrument shutdown in room 3

Computer and Instrument Shutdown

| **Activity** | **Step** | **Action** |
| --- | --- | --- |
| **CBA** | 1 | Shut down computer and then the analyzers when all runs are completed (Computer before analyzer) |
|  | 2 | Click on the **Close** button or “X” out of the program |
| **Shutdown menu** | 3 | Click on the **Start** button (Windows icon) |
|  | 4 | Next to **Restart**, click on  |
|  | 5 | Select **Shutdown** from the drop down menu |
| **CBA** | 6 | After the computer has shutdown, turn off the analyzers |

**PROCEDURE K:** Follow the steps in the table below for archiving test specimens

Archiving test specimens

| **Activity** | **Step** | **Action** |
| --- | --- | --- |
|  | 1 | Store test samples in -70⁰ C freezer, shelf 3, for approximately 3 months prior to discarding |
| **Negative samples** | 2 | Number freezer boxes 1 – 6  |
|  | 3 | Rotate boxes once filled; discard box after rotation is complete starting with box 1 |
|  | 4 | Label a box Positive Bp and Bpp samples with date range to archive positive samples |
| **Positive samples** | 5 | Indicate on cap the detected organism |
|  | 6 | Hold for 1 year |

####  METHOD PERFORMANCE

1. Clinical Sensitivity/Specificity 2: 96% / 100%
2. Analytical Sensitivity 2: *B. pertussis*:1 CFU/3 µl reaction and *B. parapertussis*: 6 CFU/3 µl reaction

**PROFICIENCY TESTING**

1. CAP *B. pertussis/B.parapertussis* (BOR), 2 shipments per year, 3 challenges each

#### ALTERNATE METHOD

1. *Bordetella pertussis* and *Bordetella parapertussis*, Molecular detection by PCR
2. Reference Lab: Mayo Medical Laboratories (Test ID: BPRP)
3. Sunquest Order code: BPPCR
4. Logistics:
	* + NP Swab in Liquid Stuart’s or Amies Charcoal transport medium
		+ Nasal wash/aspirate (0.5 ml) in sterile screw top container, no transport media
		+ Transport at RT or refrigerated : Stable up to 7 days
		+ Analytic time: 1 day
		+ Testing Monday – Friday, Sunday

## LIMITATIONS

1. Negative results do not rule out Bp and Bpp.
2. PCR detection of *B. pertussis* and *B. parapertussis* does not distinguish between viable and non-viable organism. Results should be used in conjunction with an evaluation of signs and symptoms of pertussis and available exposure information.
3. This test should not be used as a test for cure for *B. pertussis* and *B. parapertussis*.
4. This test does not distinguish between *B. pertussis* and *B. holmseii*. Some strains of *B. bronchiseptica* also contain the IS*481* gene and will cross-react at a lower level.
5. The IS1001target sequence can occasionally be found in *B. bronchiseptica* 4, 5, 6,
6. False-positive PCR results and pseudo-outbreaks have been associated specimen contamination at the point of collection from some vaccines containing *B. pertussis* DNA 6, 7, 8.
7. False-negative results can occur when low numbers of organism are present. PCR has optimal sensitivity during the first 3 weeks of cough9.
8. False negative results may occur if Bp or Bpp has genomic mutations, insertions, deletions or rearrangements.
9. Consider culture back-up during outbreak situations to rule out possible contamination9.

**REFERENCES**

1. Simplexa™ 3M™ Integrated Cycler Studio 5.0 , 3M™ Integrated Cycler Operator Manual Reference 34-8710-8382-9, PI.MOL1101.UD\_REV. F for use with user defined assays, Focus Diagnostics 2009-2012, Focus Diagnostics, Inc. Cypress, CA
2. *Bordetella* PCR Clinical Verification and Validation Study performed at Children’s Hospitals and Clinics of MN, 2015
3. Simplexa™ *Bordetella* Universal Direct Circular PI.MOL2700.IVD, Rev. F, 18-July-2012, Focus Diagnostics, Cypress, CA 90630
4. Tilley PA, Kanchana MV, Knight I, Blondeau J, Antonishyn N, Deneer H, Detection of *Bordetella pertussis* in a clinical laboratory by culture, polymerase chain reaction, direct fluorescent antibody staining; accuracy and cost, Diagn Microbiology Infect Dis. 2000 May; 37(1): 17-23.
5. Pittet LF, Emonet S, Francois P, et al, Diagnosis of Whooping cough in Switzerland: Differentiating *Bordetella pertussis* from *Bordetella holmseii* by Polymerase Chain Reaction, PLOS Feb 2014, vol 9, issue 2, e88936 pg 1-5.
6. Michael Loeffelholz, Towards Improved Accuracy of *Bordetella pertussis* Nucleic Acid Amplification Tests, Journ of Clin Micro, Volume 50, Number 7: 2186-2190
7. Mandal, Sema, Tatti KM, Woods-Stout D, Cassiday A, Faulkner E, et al, Pertussis Pseudo-outbreak linked to Specimens Contaminated by *Bordetella pertussis* DNA from Clinic Surfaces, Pediatrics; Volume 129, Number 2, Feb 2012.
8. California Department of Health – February 2011 newsletter: Pertussis: Laboratory Testing.
9. MMWR Weekly August 24, 2007/56(33); 837-842. Outbreaks of Respiratory Illness Mistakenly Attributed to Pertussis---New Hampshire, Massachusetts, and Tennessee, 2004-2006

|  |
| --- |
| **Approval** |
|  | **Approved by** | **Signature** | **Date** |
|  | Phillip Heaton PhD | Phillip R. Heaton  | 1.29.16 |
|  | Carlos Galliani MD |  |  |
|  | Patricia Ackerman, Technical Specialist | Patricia Ackerman, TS | 1.23.16 |
| **Annual Review** |
|  | Reviewed by | **Signature** | **Date** | Reviewed by | **Signature** | **Date** |
| P. Ackerman | PA | 1.23.16 |  |  |  |
|  |  |  |  |  |  |
| Historical Record |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | 1 | P. Ackerman | 1.23.16 | Initial Version |
|  |  |  |  |  |
| **Distribution** |  |  |
|  | **Location** | **# Copies** | **Location** | **# Copies** |
|  | Molecular Diagnostics rm B422 | 1 | G drive: Molecular Biology/Molecular Manual MB005.8 | 1 |